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TECH CENTER 1600/2900

## **DECLARATION**

I, Richard Craig, Ph.D., declare as follows:

- 1. I have Bachelor of Science and Master of Science Degrees in Horticulture and a Ph.D. Degree in Genetics from the Pennsylvania State University.
- I have been a member of the faculty, currently as a Professor of Plant Breeding and The J.
   Franklin Styer Professor of Horticultural Botany, at The Pennsylvania State University for 39 years.
- 3. I am the inventor or co-inventor on two Utility Patents and 24 Plant Patents on Pelargonium. A complete list of my publications and patents is attached hereto.
- 4. The following definitions are provided for reference herein:

A diploid is an organism with one pair (two copies) of cytologically identical chromosomes that can pair during prophase of meiosis. Diploids of most organisms exhibit Mendelian (disomic) inheritance. Inbred lines are easy to produce and seed production of inbred cultivars and F1 hybrids are generally possible assuming no barrier to self- or crossfertilization.

Haploid refers to the gametic chromosome number of a diploid organism. A haploid contains one-half of the number of chromosomes of the parent. In a diploid organism the haploid chromosome set is termed a **genome**; thus all of the genetic information that is representative of the organism is contained in a single genome.

A polyploid is an organism with more than one pair of each chromosome i.e., three, four, five, six or more of each chromosome is present; these chromosomes may or may not be cytologically identical. A polyploid may also be a plant of any of the above configurations that lacks or has extra individual chromosomes.

An autotetraploid is an organism with sets of four cytologically identical chromosomes that can pair during prophase of meiosis.

The flower is the site of all reproductive events in higher plants. Stamens include anthers that are the site of pollen formation. The pistil (stigma, style and ovary) is the site of maternal gamete formation. Pollination is the transfer of pollen from an anther to a stigma; fertilization is the process of gametic union.

5. A sexually reproduced plant is one that is produced from seed derived from the process of double fertilization in which a maternal egg cell (haploid) is fertilized by a paternal sperm cell (haploid) forming the diploid zygote. In a second fertilization, two fused haploid

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maternal (polar) cells are fertilized by a haploid sperm cell forming the triploid endosperm, which ultimately serves as a nutritional resource for embryo development and/or subsequent germination events.

The maternal gamete is produced in the ovule of the pistil of the flower and more specifically in the embryo sac that is connected to the maternal tissue at a site called the placenta. The products of the ovule are derived from the maternal (also known as seed, pistillate, female) parent. The most internal tissue of the embryo sac contains the megaspore mother cell. The megaspore mother cell undergoes meiosis to form four haploid megaspores; three of these megaspores disintegrate and the remaining megaspore undergoes endomitotic divisions to form an eight-nucleate (other variations are possible) embryo sac. These nuclei/cells assume specific positions in the embryo sac and three of the nuclei, including the egg cell and two polar cells, are defined by their position.

Pollen is produced in the anthers of the stamens of the flower. Pollen utilized in double fertilization can be derived from either the same parent that contributes the egg (self-fertilization), or from a genetically distinct, unrelated plant (cross-fertilization). The sperm cells are produced from pollen (microspore) mother cells via meiosis to form four microspores, a successive endomitosis to form the vegetative and generative cells, followed by an amitosis to form the two sperm cells.

The zygote undergoes successive mitotic cell divisions to form an embryo. The embryo and endosperm are contained within the embryo sac. The embryo sac is enclosed within one or two integuments; these ultimately become the seed coat (testa). The entire structure is called the ovule; thus a seed is a mature (ripened) ovule

6. Inbred lines are normally produced through self-fertilization either of naturally self-fertilized species or through controlled self-fertilization of normally cross-fertilized species. During the process of inbred line development, progeny may be selected for desirable traits of commercial and/or scientific interest. The final seed-produced progeny are uniform for these traits.

Self-fertilization leads to genetic homozygosity (uniformity of alleles at a gene). With each generation of self-fertilization, heterozygosity of plants and genes (each and all genes) is decreased by 50%; conversely homozygosity is increased proportionally. After a certain number (5-7) of generations of self-fertilization, homozygosity of loci and plants approaches 100 % and progeny are phenotypically uniform for most traits.

Breeding progress (with the exception of homozygosity) may be impeded or affected by such phenomena as selection, epistasis (interaction of at least two genes affecting a single trait), linkage (genes segregating together), cytoplasmic inheritance (maternal or paternal influences are not caused by nuclear genes but by genetic elements in chloroplasts or mitochondria), and environmental influences.

Inbred lines may be used as parents of F1 hybrid cultivars. Inbred lines may also be used for inheritance as well as gene mapping studies.

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It is crucial to recognize that not all species can be self-fertilized (or continually self-fertilized), that is, in many species inbred lines are not possible either commercially or scientifically. Reasons for lack of ability to self-fertilize may be dioecy (maternal and paternal gametes produced on separate plants), inbreeding depression (the loss of vigor or fertility due to self-fertilization), self-incompatibility (viable pollen which is incapable of fertilizing a plant with similar "incompatibility" alleles), or various gametic and/or zygotic sterilities. In these cases only cross-fertilizations are usually possible.

- 7. An F1 hybrid is the sexually reproduced progeny developed from the cross-fertilization of two inbred parents (true breeding and genetically homozygous for traits of commercial and/or scientific interest). The parents usually have different genetic (allelic) constitutions and the F1 hybrids are heterozygous for all genes that are polymorphic between the two parents. In contrast to the genetic heterozygosity of F1 plants, the progeny thereof are homogeneous in appearance because they share the same genetic constitution. Conversely F1 hybrids will not produce uniform progeny upon self- or cross-fertilization. All genes that are heterozygous in the F1 hybrids (polymorphic between the original parents) segregate in the succeeding generations. Once an F1 hybrid is created, it may also be asexually propagated and cloned.
  - 8. The term hybrid can be applied to any sexually reproduced progeny resulting from the cross-fertilization of two or more parents regardless of the origin or genetic constitution of those parents. The parents may be of different genera (intergeneric hybrids), different species (interspecific hybrids), different botanical varieties (intervarietal hybrids), different cultivars, different breeding lines, etc. Hybrids result from double fertilization with the sperm cells (pollen) contributed by one parent and the egg cells contributed by a second parent. With the exception of F1 hybrids and inbred lines, cross-fertilization and indeed self-fertilization of most plants produces heterozygous and heterogeneous progeny. In most instances, every progeny will be different from every other both genotypically (genetic constitution) and phenotypically (traits).

This non-uniformity is normal in species that are **diploid.** Thus, when one makes a cross-fertilization of heterozygous parents, one cannot predict the specific combination of traits in the progeny. When a large number of genes have different allelic combinations in the parents, the possible genotypic combinations in the hybrid progeny approach infinity.

Such genotypic diversity among hybrids is magnified when species are polyploid - having more than two identical genomes. Thus, each gene is present in more than two copies leading to an exponential number of phenotypes in the progeny. Many fruit and ornamental plants are polyploid in origin and indeed are polyploid in cultivation. This polyploidy often is accompanied by positive phenotypic characteristics such as larger fruit, improved keeping quality, etc. When these species are cross or self-fertilized, they produce highly heterozygous and heterogeneous progeny. The possible combination of genes is infinite.

For these reasons, polyploidy and genetic heterozygosity, many cultivars can only be propagated (replicated, cloned) through asexual processes for commercial production.

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- 9. An asexually reproduced plant is produced from cells, tissues, or organs of a mother plant without the process of fertilization. Asexual propagation may occur through cuttings with adventitious roots, physical divisions, runners, layering, grafting, tissue culture, bulbs, corms, tubers, adventitious embryony, and apomixis. Adventitious embryony is the production of embryos from somatic tissues; these are entirely maternal in origin and genetic constitution. Apomixis (parthenogenesis) is the asexual production of seed from solely maternal tissues via specialized processes. When a single progeny or an infinite number of progeny are asexually produced directly from a single mother plant, this is defined as cloning and the progeny are termed clones. With the rare exception of somatic mutations all clones share the same genetic constitution, and are exactly identical to the mother plant.
- 10. Starting only from a photograph or a written description of a particular cultivar, a plant breeder cannot reproduce the cultivar. No person can independently create through fertilization and hybridization the exact genetic replica of another plant.

Without access to the actual desired plant (or an actual plant of an inbred cultivar or the inbred parents of an F1 hybrid cultivar), the only route to recreating the particular cultivar is to self- or cross-fertilize various parental plants until the desired genotype is somehow recreated. The number of combinations of genes and resulting genotypes from hybridization of diverse parents approaches infinity. Even when the parents of the desired cultivar are known (i.e. the starting materials for recreating the cultivar are known), hybridization thereof still involves the potential for essentially infinite combinations of genes. Hence, such an attempt to recreate a particular cultivar is futile.

11. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Richard Craig, Ph.D.

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1/5/02 Date